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Novel Small-Molecule Antibacterial Agents

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5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS
Yuan-Ping Pang

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
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Rochester, MN 55905 -0002

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U.S. Army Research Office
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Research Triangle Park, NC 27709-2211

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56085-LS.6

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13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT
The specific aim of this proposal is to develop improved small-molecule botulinum neurotoxin serotype A endopeptidase (BoNTAe) inhibitors with Ki values of <100 nM. We have developed BoNTAe inhibitors MHC and HAB (see US Patent 8,404,728 B2) that showed significant 6-hour-post-exposure protection of mice against 5 LD50 BoNTA. HAB also showed significant 4-hour-post-exposure protection of zebrafish against 5 LD50 BoNTA. Our kinetics and affinity analyses using the surface plasmon resonance technique showed that the Ki value of our BoNTAe inhibitor AHP (see US Patent 8,404,728 B2) is 71 ± 26 nM (2 independent experiments with chi square

15. SUBJECT TERMS
Small Molecules, Reversible Inhibitors, Irreversible Inhibitors, Therapeutics, Antidotes, Countermeasures, Botulism, and Neurotoxins.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Yuan Pang
UU	UU	UU	UU		19b. TELEPHONE NUMBER 507-266-7991

Report Title

Novel Small-Molecule Antibacterial Agents

ABSTRACT

The specific aim of this proposal is to develop improved small-molecule botulinum neurotoxin serotype A endopeptidase (BoNTAe) inhibitors with Ki values of <100 nM. We have developed BoNTAe inhibitors MHC and HAB (see US Patent 8,404,728 B2) that showed significant 6-hour-post-exposure protection of mice against 5 LD50 BoNTA. HAB also showed significant 4-hour-post-exposure protection of zebrafish against 5 LD50 BoNTA. Our kinetics and affinity analyses using the surface plasmon resonance technique showed that the Ki value of our BoNTAe inhibitor AHP (see US Patent 8,404,728 B2) is 71 ± 26 nM (2 independent experiments with chi square values of 0.393 and 0.396). In addition, we have developed a generic approach to cysteine-targeting irreversible inhibitors of pathogenic enzymes (Adv. Insect Physiol. 46, 435–494, 2014) that enables convertion of our reversible BoNTAe inhibitors to irreversible inhibitors that target Cys164 in the BoNTAe active site to effectively counteract BoNTA that has an unusually long in vivo half life of ~31 days.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
10/02/2012	4.00	Yuan-Ping Pang, Jon Davis, Shaohua Wang, Jewn Giew Park, Madhusoodana P. Nambiar, James J. Schmidt, Charles B. Millard. Small Molecules Showing Significant Protection of Mice against Botulinum Neurotoxin Serotype A, PLoS ONE, (04 2010): 0. doi: 10.1371/journal.pone.0010129
11/16/2009	3.00	Y. Pang, A. Vummenthala, R. Mishra, J. Park, S. Wang, J. Davis, C. Millard, J. Schmidt. Potent New Small-Molecule Inhibitor of Botulinum Neurotoxin Serotype A Endopeptidase Developed by Synthesis-Based Computer-Aided Molecular Design, , (11 2009): . doi:
TOTAL:	**2**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
09/09/2009	1.00	Y. Pang, A. Vummenthala, R. Mishra, J. Park, S. Wang, J. Davis, C. Millard, J. Schmidt. Potent New Small-Molecule Inhibitor of Botulinum Neurotoxin Serotype A Endopeptidase Developed by Synthesis-Based Computer-Aided Molecular Design, (09 2009)
10/20/2009	2.00	Y. Pang, A. Vummenthala, R. Mishra, J. Park, S. Wang, J. Davis, C. Millard, J. Schmidt. Potent New Small-Molecule Inhibitor of Botulinum Neurotoxin Serotype A Endopeptidase Developed by Synthesis-Based Computer-Aided Molecular Design, (10 2009)
TOTAL:	**2**	

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

07/01/2014 5.00 Yuan-Ping Pang. Insect Acetylcholinesteraseas a Target for Effective and Environmentally Safe Insecticides, Adv. Insect Physiol.: Elsevier, (04 2014)

TOTAL: **1**

Patents Submitted

Patents Awarded

Small-Molecule Botulinum Toxin Inhibitors, Yuan-Ping Pang, et al., US Patent number: 8,404,728 B2 (granted Mar 26, 2013).

Awards

Graduate Students

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Post Doctorates

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Yuan-Ping Pang	0.10	
FTE Equivalent:	**0.10**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Miranda Ming-Wai So	1.00	BS
FTE Equivalent:	**1.00**	
Total Number:	**1**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 1.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 1.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 1.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 1.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 1.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:..... 1.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Jewn Giew Park	0.45
FTE Equivalent:	**0.45**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

5 **Small-molecule botulinum toxin inhibitors**

Patent Filed in US? (5d-1) Y

Patent Filed in Foreign Countries? (5d-2) N

Was the assignment forwarded to the contracting officer? (5e) Y

Foreign Countries of application (5g-2):

5a: Yuan-Ping Pang

5f-1a: Mayo Clinic

5f-c: 200 First Street SW

Rochester MN 55905

5a: Yuan-Ping Pang

5f-1a: Mayo Clinic

5f-c: 200 First Street SW

Rochester MN 55905

Scientific Progress

The specific aim of this proposal is to develop improved small-molecule botulinum neurotoxin serotype A endopeptidase (BoNTAe) inhibitors with Ki values of <100 nM. We have developed BoNTAe inhibitors MHC and HAB (see US Patent 8,404,728 B2) that showed significant 6-hour-post-exposure protection of mice against 5 LD50 BoNTA. HAB also showed significant 4-hour-post-exposure protection of zebrafish against 5 LD50 BoNTA. Our kinetics and affinity analyses using the surface plasmon resonance technique showed that the Ki value of our BoNTAe inhibitor AHP (see US Patent 8,404,728 B2) is 71 ± 26 nM (2 independent experiments with chi square values of 0.393 and 0.396). In addition, we have developed a generic approach to cysteine-targeting irreversible inhibitors of pathogenic enzymes (Adv. Insect Physiol. 46, 435–494, 2014) that enables convertion of our reversible BoNTAe inhibitors to irreversible inhibitors that target Cys164 in the BoNTAe active site to effectively counteract BoNTA that has an unusually long in vivo half life of ~31 days.

Technology Transfer